EVALUATION OF ANTI-CARIOGENIC PROPERTY OF GREEN TEA, STABILIZED CHLORINE DIOXIDE AND CHLORHEXIDINE BY ASSESSING ITS EFFECT ON PH OF DENTAL PLAQUE –A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Background

Dental plaque is the primary source for dental caries and there is no proper vaccine that can affect dental plaques.

Objective

Daily use of an efficient anti-plaque product can be very beneficial in plaque control and thus, prevention of caries. Thus, this study aimed to evaluate the anti-cariogenic potential of mouth rinse containing green tea extract, 0.1% stabilized chlorine dioxide and 0.2% chlorhexidine on pH of dental plaque, over a period of 30 minutes and after 10% sucrose challenge.

Materials and Methods

A randomized control trial was conducted among 48 patients aged 18 to 30 years visiting the department of conservative dentistry and endodontics, Mahae Institute of dental sciences and hospital, Mahae and were randomly assigned into one of the four groups. Student's test and analysis of variance (One Way ANOVA) were used for analysis.

Results

The group I showed maximum pH 7.49 after 10 mins and pH 6.68 after sucrose challenge. Group II showed a maximum pH of 6.82after 30 minutes and after sucrose challenge pH drastically reduced after 5 minutes and results were statistically significant (P<0.005). Group III showed a maximum pH of 6.93at 10 minutes and after sucrose challenge values showed statistically significant (P<0.005). Group IV showed no statistically significant values at different intervals.

Conclusions

The present study concluded that the green tea extract, stabilized chlorine dioxide and chlorhexidine have anti-cariogenic potential. All the groups have showed significant effectiveness in maintaining pH and preventing fall of pH below the critical value after sucrose challenge.

KEYWORDS: Dental Caries, Mouth Rinses, Plaque

INTRODUCTION OR BACK GROUND

Dental caries is the most rampant chronic disease affecting the humankind. Once it occurs its manifestation persist throughout the life even though the lesion is treated. Dental caries, a disease caused by the complex interaction of food, especially starches and sugars, with the bacteria that form dental plaque. The word also refers to the tooth cavities that result from the infection.[1] Microorganisms produce acids that cause demineralization of enamel and enzymes that attack the protein component of the tooth. This development, if untreated, finally leads to the formation of deep cavities and microbial infection of the pulp chamber, which contains blood vessels and nerves. [1,2]

The control of dental caries presence, one of the greatest challenges that must be met by the dental profession. There is a feeling that the treatment aspect of the disease is overemphasized and preventive aspect is neglected. With our present understanding of the etiology and prevention of the dental caries we can markedly reduce the tooth decay in most populations.

Green tea, a common beverage, has established use in medicine and has also got antibacterial activity against microorganisms comprising dental plaque. [3]This action is attributed to the presence of catechins (Polyphenols) which have properties in-vitro that suggest an anti- cariogenic activity.[1,3]

Chlorhexidine gluconate (Chlorhexidine) is a wide spectrum antimicrobial remedy. Substitute as an antiseptic, it is an effective bactericidal agent against all categories of microbes, including bacterial, yeast, and viruses[2]. Chlorhexidine, an antimicrobial agent that can suppress the growth of Mutans streptococci, has been considered as having the potential to prevent dental caries. For patients with a high risk of caries, Chlorhexidine rinses can be successfully used to reduce the number of pathogenic bacteria. This should be integrated into an intensive home care program to maintain the optimum level of oral hygiene. [2]

Chlorine dioxide is a highly reactive molecule and has strong antimicrobial properties, ease of use and has widespread availability. It is a free radical and cannot be stabilized as a gas or in solution or gel. The compound called "stabilized chlorine dioxide" by some manufacturers, is in fact, sodium chlorite, although the phrase is too often used as synonymous with chlorine dioxide. The "stabilized" term attempts to describe a formulation which has the same or similar chemical properties to the now familiar disinfectant, chlorine dioxide, and its many applications. Chemically this disguise would be similar to calling sodium chloride table salt, "stabilized chlorine." Chlorine Dioxide (ClO2) is a small, volatile and highly energetic molecule derived from chlorine and being used in food processing industry, dental waterline treatment and also surface disinfection. [3]Thus this present study aims to evaluate the anti-cariogenic potential of mouth rinses containing green tea extract, 0.1% stabilized chlorine dioxide and 0.2% chlorhexidine by studying its effect on pH of dental plaque over a period of 30 minutes and after 10% sucrose challenge.

MATERIALS AND METHODS

A randomized control trail was conducted over a period of 3 months from January 2016 to march 2016. The sample size was calculated to be 12 in each group with a statistical power of 80% at 5% level of significance. The ethical approval for the study was obtained from the institutional review board before the commencement of the study and a total of forty-eight patients attending OPD (outpatient department) of the department of conservative dentistry and endodontics, Mahae Institute of dental sciences and hospital, Mahae, were selected and randomly allocate into one of the four groups. Consent forms were prepared in English and local language, Kannada. Inclusion criteria for the study were

patients with DMFT score ≤ 5 and patients aged 18-30 years and above. The exclusion criteria for the study were patients with an any significant systemic diseases like uncontrolled hypertension, diabetes, patients with history of radiation therapy and pregnancy. After taking the informed consent, subjects satisfying the inclusion criteria are randomly divided into four groups: GROUP I - 0.5% green tea extract mouth rinse group, GROUP II - 0.1% stabilized chlorine dioxide mouth rinse group, GROUP III - 0.2% chlorhexidine mouth rinse group and GROUP IV - controlled group or the distilled water mouth rinse group.

Preparation of Green Tea Extracts Mouthwash and 10% Sucrose Solution

For preparing 100 ml of 0.5% green tea mouthwash, 500 mg of green tea extract powder was weighed over an electronic weighing machine and mixed with 100 ml of distilled water. The mixture is then heated over a flame till it boils and allowed it to cool at room temperature then the mixture is filtered with the help of a filter paper.[3]

Two different types of commercially available mouthwashes were used in this study which included 0.2% chlorhexidine mouthwash (Hexidine) and 0.1% stabilized chlorine dioxide (Freshclor).

Initial recording of pH of patient's dental plaque and saliva was done in all the groups. Dental plaque was collected with an autoclaved periodontal probe from any teeth, any surface except occlusal surface. Plaque pH was evaluated with the help of special pH strips. Subjects were allowed to rinse the mouth for 5 minutes with 10 ml of 0.5% green tea (group 1), 0.1% stabilized chlorine dioxide (group 2), 0.2% chlorhexidine (group 3), distilled water (group 4), at least 2 hours after eating or drinking.

After 10, 20, and 30 minutes, pH of plaque and saliva was measured, after that subjects were instructed to rinse their mouths for 2 minutes with 10 ml of 10% sucrose solution. Plaque samples were collected after 0, 5, 10, 20, and 30 minutes and pH was recorded.

All the Statistical analysis was carried out using the statistical package for social sciences software, SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). P = 0.05 was considered as statistically significant. Student 't' test and ANOVA were used.

Findings

**Table 1: Mean Plaque pH score after using Green Tea
Mouth Rinses at different Time Intervals**

Group	Observation	Mean(SD)	F value	P value
Group I – Green Tea	Pre rinse	6.74 (0.25)	2.148	0.765[NS]
	Post rinse 10 min	7.49(0.62)		
	Post rinse 20 min	6.78(0.24)		
	Post rinse 30 min	6.85(0.19)		
	Post 10% sucrose 0 min	6.54(0.37)	6.830	0.000*
	Post 10% sucrose 5 min	6.23(0.22)		
	Post 10% sucrose 10 min	6.29(0.16)		
	Post 10% sucrose 20 min	6.51(0.19)		
	Post 10% sucrose 30 min	6.68(0.23)		

*Statistically significant [NS]– Not significant

Table 1 shows the comparison of green tea at different intervals. Maximum pH 7.49 was shown at post rinse after 10 minutes (figure 1) after sucrose challenge green tea showed ph of 6.68 after 30min. **[Figure 2]**

Table 2: Mean Plaque pH Score after using 0.1% Stabilized
Chlorine dioxide at different Time Intervals

Group	Observation	Mean	F value	P value
Group II – **0.1% Stabilized** **Chlorine dioxide**	Pre rinse	6.785(0.282)	0.73	0.974
	Post rinse 10 min	6.770(0.530)		
	Post rinse 20 min	6.785(0.145)		
	Post rinse 30 min	6.829(0.193)		
	Post 10% sucrose o min	6.744(0.259)	23.62	0.000
	Post 10% sucrose 5 min	6.097(0.206)		
	Post 10% sucrose 10 min	6.231(0.278)		
	Post 10% sucrose 20 min	6.611(0.176)		
	Post 10% sucrose 30 min	6.758(0.133)		

*Statistically significant NS – Not significant

Table 2: comparison of 0.1% Stabilized Chlorine dioxide at different intervals and shows maximum pH of 6.829 post rinse 30 minutes. After sucrose challenge values shows statistically significant ($p < 0.005$).

Table 3: Mean Plaque pH Score after 0.2% Chlorhexidine Rinse at different Time Intervals

Group	Observation	Mean	F value	P value
Group III – **0.2% Chlorhexidine**	Pre rinse	6.869(0.259)	0.200	0.896[NS]
	Post rinse 10 min	6.931(0.368)		
	Post rinse 20 min	6.857(0.171)		
	Post rinse 30 min	6.878(0.158)		
	Post 10% sucrose o min	6.742(0.177)	11.916	0.000*
	Post 10% sucrose 5 min	6.217(0.193)		
	Post 10% sucrose 10 min	6.366(0.338)		
	Post 10% sucrose 20 min	6.670(0.235)		
	Post 10% sucrose 30 min	6.759(0.249)		

*Statistically significant   NS – Not significant

Table 3 shows comparison of 0.2% Chlorhexidine rinse group at different intervals maximum pH of 6.931 post rinse 30 minutes. After sucrose challenge values shows statistically significant ($p < 0.005$).

Table 4: Mean Plaque pH Score after using the Distilled
Water as a Control at different Time Interval

Group	Observation	Mean	F value	P value
Group IV – **Distilled Water**	Pre rinse	7.064(0.135)	0.560	0.756[NS]
	Post rinse 10 min	7.140(0.223)		
	Post rinse 20 min	7.007(0.052)		
	Post rinse 30 min	6.905(0.236)		
	Post 10% sucrose o min	6.002(0.257)	0.804	0.971[NS]
	Post 10% sucrose 5 min	6.179(0.103)		
	Post 10% sucrose 10 min	6.212(0.133)		
	Post 10% sucrose 20 min	6.234(0.114)		
	Post 10% sucrose 30 min	6.231(0.301)		

*Statistically significant   NS – Not significant

Table 4 shows comparison of distilled water group at different intervals maximum pH of 7.140 post rinse 10 minutes and results were not statistically significant.

Table 5: Mean Plaque Score for Four Groups after Giving the Mouth Rinses

Observation	Groups	Mean	F value	P value
Pre Rinse	Green Tea	6.748(0.25)	1.342	0.279[NS]
	0.1% Stabilized Chlorine dioxide	6.785(0.28)		
	0.2% Chlorhexidine rinse group	6.869(0.25)		
	Control Group (Distilled Water)	6.937(0.22)		
Post rinse 10 min	Green Tea	6.490(0.62)	2.265	0.094[NS]
	0.1% Stabilized Chlorine dioxide	6.770(0.53)		
	0.2% Chlorhexidine rinse group	6.931(0.36)		
	Control Group (Distilled Water)	6.904(0.22)		
Post rinse 20 min	Green Tea	6.789(0.24)	1.399	0.256[NS]
	0.1% Stabilized Chlorine dioxide	6.785(0.14)		
	0.2% Chlorhexidine rinse group	6.857(0.17)		
	Control Group (Distilled Water)	6.930(0.22)		
Post rinse 30 min	Green Tea	6.850(0.19)	0.560	0.644[NS]
	0.1% Stabilized Chlorine dioxide	6.829(0.19)		
	0.2% Chlorhexidine rinse group	6.878(0.15)		
	Control Group (Distilled Water)	6.925(0.21)		

[*]**Statistically significant, [NS]- Not significant**

Table 5 shows mean plaque score for four groups after giving the mouth rinses. It was observed that green tea and 0.2% chlorhexidine group showed a similar reduction in plaque Ph compared to other two group and the results were statistically significant.

Table 6: Mean Plaque Score for Four Groups after Giving 10% Sucrose Challenge

Observation	Groups	Mean	F value	P-Value
Post rinse 0 min	Green Tea	6.540(0.37)	3.026	0.039*
	0.1% Stabilized Chlorine dioxide	6.744(0.25)		
	0.2% Chlorhexidine rinse group	6.742(0.177)		
	Control Group (Distilled Water)	6.860(0.19)		
Post rinse 5 min	Green Tea	6.230(0.22)	60.579	0.000*
	0.1% Stabilized Chlorine dioxide	6.097(0.20)		
	0.2% Chlorhexidine rinse group	6.217(0.19)		
	Control Group (Distilled Water)	5.3050(0.15)		
Post rinse 10 min	Green Tea	6.2933(0.16)	17.613	0.000*
	0.1% Stabilized Chlorine dioxide	6.2317(0.27)		
	0.2% Chlorhexidine rinse group	6.3667(0.33)		
	Control Group (Distilled Water)	5.690(0.19)		
Post rinse 20 min	Green Tea	6.5158(0.19)	18.107	0.000*
	0.1% Stabilized Chlorine dioxide	6.611(0.17)		
	0.2% Chlorhexidine rinse group	6.670(0.23)		
	Control Group (Distilled Water)	6.123(0.19)		
Post rinse 30 min	Green Tea	6.689(0.23)	1.360	0.267[NS]
	0.1% Stabilized Chlorine dioxide	6.758(013)		
	0.2% Chlorhexidine rinse group	6.759(0.24)		
	Control Group (Distilled Water)	6.606(0.22)		

*Statistically significant, [NS]- Not significant

Table 6 shows mean plaque score for four groups after giving 10% sucrose challenge where it was observed that green tea showed equal effectiveness compared to 0.2% chlorhexidine group.

DISCUSSIONS

Management of dental caries has evolved from a centuries old surgical model to the present medical model. This shift is because of the change in the outlook of the dental profession towards dental caries. Dental caries is now being viewed in dual perspective- "caries as a disease" and "caries as a lesion". The surgical model of management of dental "caries as a lesion", tooth preparation, and restoration, is now being reserved for the lesion that has cavitated and penetrated the dentinal aspect of tooth structure. The medical model of management of dental caries as a disease includes (a) identifying the risk group for the disease; (b) remineralizing the non-cavitated carious tooth, without surgical intervention; and (c) preventing the occurrence and recurrence of the disease in the individual.[4]

The exponential advancements in the field of cariology have reemphasized the importance of prevention. Fluoride has been the most popular preventive material because of its ability to remineralize the tooth and make it acid resistant. Causation of dental caries is multifactorial, involving dietary factors, salivary factors, and microbial factors; therefore, other preventive materials have emerged, targeting these causative factors. For example, the use of antimicrobial agents, like chlorhexidine, is now recognized as yet another useful preventive measure.

Indian civilization, as everyone is aware, is very ancient and rich in resources. The use of herbs and plants for treating diseases has been common practice here since ages. Review of literature reveals abundant evidence for the use of plant and plant products in preventing caries.[5]Green tea catechins have proven health benefits in medical conditions where it is reputed to be helpful against various conditions such as cancer, rheumatoid arthritis, high cholesterol levels, cardiovascular diseases, infection, impaired immune function, to name a few.

There is now a solid body of scientific evidence that strongly suggests that green tea catechins may exert a significant anti-cariogenic effect, by virtue of various activities against α-hemolytic streptococci. These include: a direct bactericidal effect against streptococcus mutans; prevention of bacterial adherence to teeth; inhibition of glucosyl transferase, thus limiting the biosynthesis of sticky glucan; inhibition of human and bacterial amylases [4].

The use of chlorhexidine for caries prevention has been a controversial topic among dental educators and clinicians. In several reviews, it has been concluded that most persistent reduction of mutans streptococci have been achieved by chlorhexidine varnishes, followed by gels and, lastly, mouth rinses. Chlorhexidine, an antimicrobial agent that can suppress the growth of mutans streptococci, has been considered as having the potential to prevent dental caries.[6]

Chlorine dioxide is used as a disinfectant at concentrations of 0.1-15mM, but an effective clinical concentration has not been determined. Stabilized Chlorine Dioxide is registered with the EPA (Environmental Protection Agency) as an excellent bactericide, fungicide and antimicrobial agent. Molecular Chlorine dioxide released from aqueous solution shown to have bacteriostatic and bacteriocidal effects against aerobic, anaerobic and facultative micro- organisms.[3]

The concentration of green tea extract used in this study was 0.5%. Kaneko et al,(1993) found that the anti-plaque effect of rinsing the mouth with 20 ml of tea catechin 0.25% solution persisted for upto 90 minutes. [5]In this study the pH of plaque and saliva were recorded after 10 min, 20 min and 30 min to find out how the effect of mouthwash varied over a period of time and also for the sake of convenience.

On the other hand, the sucrose challenge was given 30 min after the green tea extract mouthwash based on the study of Hirasawa et al, (2006) in which the effect of the interval between rinsing with 0.25 mg/ml green tea catechins and rinsing with 10% sugar on plaque pH at various assay times in vivo was measured[4]. More inhibition was observed after a

30 min interval between rinses than after 2-min or 120-min intervals. The inhibition of plaque pH falls by green tea catechins after too short (2 min) or too long (120 min) an interval was not satisfactory. This finding suggested that the reaction between green tea catechins and dental plaque needs a suitable interval for penetration of catechins into the dental plaque.

CONCLUSIONS

The result of this study clearly indicates that green tea extract is very effective in increasing the pH of dental plaque until sucrose challenge was given.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: Obtained

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